Claims

1. A screening method for a somatic cell nuclear reprogramming substance, which comprises the following steps (a) and (b):
(a) a step for bringing into contact with each other a somatic cell comprising a gene wherein a marker gene is present at a position permitting expression control by the expression control region of an ECAT gene, and a test substance,
(b) a step following the aforementioned step (a), for determining the presence or absence of the emergence of cells expressing the marker gene, and selecting a test substance allowing the emergence of the cells as a somatic cell nuclear reprogramming substance candidate.

2. The screening method of claim 1, wherein the ECAT gene is one or more genes selected from among the ECAT1 gene, ECAT2 gene, ECAT3 gene, ECAT4 gene, ECAT5 gene, ECAT6 gene, ECAT7 gene, ECAT8 gene, ECAT9 gene and Oct3/4 gene.

3. The screening method of claim 1 or 2, wherein the marker gene is a drug resistance gene, a fluorescent protein gene, a luminescent enzyme gene, a chromogenic enzyme gene or a gene comprising a combination thereof.

4. The screening method of any of claims 1 to 3, wherein the somatic cell is a somatic cell comprising a gene resulting from knocking in the marker gene to the ECAT gene.

5. The screening method of claim 4, wherein the somatic cell is a somatic cell homozygously comprising the gene resulting from knocking in the marker gene to the ECAT gene.

6. The screening method of claim 4 or 5, wherein the ECAT gene is one or more genes selected from among the ECAT1 gene, ECAT2 gene, ECAT3 gene, ECAT4 gene, ECAT5 gene, ECAT6 gene, ECAT7 gene, ECAT8 gene, ECAT9 gene and Oct3/4 gene.

7. The screening method of claim 1, which comprises the following steps (a) and (b):
(a) a step for bringing into contact with each other a somatic cell comprising a gene resulting from knocking in a gene comprising a drug resistance gene to the ECAT2 gene, and a test substance,
(b) a step following the aforementioned step (a), for determining the presence or absence of surviving cells in a selection medium, and selecting a test substance allowing the emergence of the surviving cells as a somatic cell nuclear reprogramming substance candidate.

8 The screening method of claim 1, which comprises the following steps (a) and (b):
(a) a step for bringing into contact with each other a somatic cell comprising a gene resulting from knocking in a gene comprising a drug resistance gene to the ECAT3 gene, and a test substance,
(b) a step following the aforementioned step (a), for determining the presence or absence of surviving cells in a selection medium, and selecting a test substance allowing the emergence of the surviving cells as a somatic cell nuclear reprogramming substance candidate.

9. The screening method of claim 1, which comprises the following steps (a) and (b):
(a) a step for bringing into contact with each other a somatic cell comprising a gene resulting from knocking in a gene comprising a drug resistance gene to the ECAT5 gene, and a test substance,
(b) a step following the aforementioned step (a), for determining the presence or absence of surviving cells in a selection medium, and selecting a test substance allowing the emergence of the surviving cells as a somatic cell nuclear reprogramming substance candidate.

10. The screening method of claim 1, which comprises the following steps (a) and (b):
(a) a step for bringing into contact with each other a somatic cell comprising genes resulting from knocking in a gene comprising a drug resistance gene to each of the ECAT2 gene and ECAT3 gene, and a test substance,
(b) a step following the aforementioned step (a), for determining the presence or absence of surviving cells in a selection medium, and selecting a test substance allowing the emergence of the surviving cells as a somatic cell nuclear reprogramming substance candidate.

11. The screening method of claim 10, wherein the different drug resistance genes have been knocked in to ECAT2 gene and the ECAT3 gene.

12. The screening method of any of claims 7 to 11, wherein the somatic cell is a somatic cell homozygously comprising a gene resulting from knocking in a gene comprising a drug resistance gene to an ECAT gene.

13. The screening method of claim 1, which comprises the following steps (a) and (b):
(a) a step for bringing into contact with each other a somatic cell comprising a gene resulting from knocking in a gene comprising a drug resistance gene to the ECAT4 gene, and a test substance,
(b) a step following the aforementioned step (a), for determining the presence or absence of surviving cells in a selection medium, and selecting a test substance allowing the emergence of the surviving cells as a somatic cell nuclear reprogramming substance candidate.

14. The screening method of claim 13, wherein the somatic cell is a somatic cell heterozygously comprising a gene resulting

from knocking in a gene comprising a drug resistance gene to the ECAT4 gene.

15. The screening method of claim 13, which comprises the following steps (a) and (b):
(a) a step for supplying ECAT4 to a somatic cell comprising a gene resulting from knocking in a gene comprising a drug resistance gene to the ECAT4 gene, and bringing it into contact with a test substance,
(b) a step following the aforementioned step (a), for determining the presence or absence of surviving cells in a selection medium, and selecting a test substance allowing the emergence of the surviving cells as a somatic cell nuclear reprogramming substance candidate.

16. The screening method of claim 15, wherein the somatic cell is a somatic cell homozygously comprising a gene resulting from knocking in a gene comprising a drug resistance gene to the ECAT4 gene.

17. A nuclear reprogramming substance selected using the screening method of any of claims 1 to 16.

18. The nuclear reprogramming substance of claim 17, which is a gene or protein derived from ES cells.

19. The nuclear reprogramming substance of claim 18, wherein the ES cell is an ES cell with the NAT1 gene destroyed.

20. A substance derived from ES cells with the NAT1 gene destroyed.

21. The substance of claim 20, which is a cDNA library, a protein library, or a cell extract.

22. A use of a knock-in mouse comprising a gene resulting from

knocking in a marker gene to an ECAT gene as a source of the somatic cell used in the screening method of any of claims 1 to 16.

23. The use of claim 22, wherein the knock-in mouse is a knock-in mouse homozygously comprising a gene resulting from knocking in a marker gene to an ECAT gene.

24. The use of claim 22 or 23, wherein the ECAT gene is one or more genes selected from among the ECAT1 gene, ECAT2 gene, ECAT3 gene, ECAT4 gene, ECAT5 gene, ECAT6 gene, ECAT7 gene, ECAT8 gene, ECAT9 gene and Oct3/4 gene.

25. The use of any of claims 22 to 24, wherein the marker gene is a drug resistance gene, a fluorescent protein gene, a luminescent enzyme gene, a chromogenic enzyme gene or a gene comprising a combination thereof.

26. A somatic cell comprising a gene wherein a marker gene is present at a position permitting expression control by the expression control region of an ECAT gene.

27. The somatic cell of claim 26, wherein the ECAT gene is one or more genes selected from among the ECAT1 gene, ECAT2 gene, ECAT3 gene, ECAT4 gene, ECAT5 gene, ECAT6 gene, ECAT7 gene, ECAT8 gene, ECAT9 gene and Oct3/4 gene.

28. The somatic cell of claim 26 or 27, wherein the marker gene is a drug resistance gene, a fluorescent protein gene, a luminescent enzyme gene, a chromogenic enzyme gene or a gene comprising a combination thereof.

29. The somatic cell of any of claims 26 to 28, which comprises a gene resulting from knocking in a marker gene to an ECAT gene.

30. The somatic cell of claim 29, which homozygously comprises a gene resulting from knocking in a marker gene to an ECAT gene.

31. The somatic cell of claim 30, which is a differentiated ES cell homozygously comprising a gene resulting from knocking in a marker gene to the ECAT4 gene.

32. The somatic cell of claim 31, into which ECAT4 has been supplied.

33. A selection method for ES-like cells, which comprises the following steps (a) and (b):
(a) a step for bringing into contact with each other a somatic cell comprising a gene wherein a marker gene is present at a position permitting expression control by the expression control region of an ECAT gene, and a somatic cell nuclear reprogramming substance,
(b) a step following the aforementioned step (a), for selecting cells expressing the marker gene as ES-like cells.

34. The selection method of claim 33, wherein the ECAT gene is one or more genes selected from among the ECAT1 gene, ECAT2 gene, ECAT3 gene, ECAT4 gene, ECAT5 gene, ECAT6 gene, ECAT7 gene, ECAT8 gene, ECAT9 gene and Oct3/4 gene.

35. The selection method of claim 33 or 34, wherein the marker gene is a drug resistance gene, a fluorescent protein gene, a luminescent enzyme gene, a chromogenic enzyme gene or a gene comprising a combination thereof.

36. The selection method of claim 33, which comprises the following steps (a) and (b):
(a) a step for bringing into contact with each other a somatic cell comprising a gene wherein a drug resistance gene is present at a position permitting expression control by the

expression control region of the ECAT2 gene, and a somatic cell nuclear reprogramming substance,
(b) a step following the aforementioned step (a), for selecting surviving cells in a selection medium as ES-like cells.

37. The selection method of claim 33, which comprises the following steps (a) and (b):
(a) a step for bringing into contact with each other a somatic cell comprising a gene wherein a drug resistance gene is present at a position permitting expression control by the expression control region of the ECAT3 gene, and a somatic cell nuclear reprogramming substance,
(b) a step following the aforementioned step (a), for selecting surviving cells in a selection medium as ES-like cells.

38. The selection method of claim 33, which comprises the following steps (a) and (b):
(a) a step for bringing into contact with each other a somatic cell comprising a gene wherein a drug resistance gene is present at a position permitting expression control by the expression control region of the ECAT5 gene, and a somatic cell nuclear reprogramming substance,
(b) a step following the aforementioned step (a), for selecting surviving cells in a selection medium as ES-like cells.

39. The selection method of claim 33, which comprises the following steps (a) and (b):
(a) a step for bringing into contact with each other a somatic cell comprising genes wherein a drug resistance gene is present at a position permitting expression control by the expression control region of each of the ECAT2 gene and the ECAT3 gene, and a somatic cell nuclear reprogramming substance,
(b) a step following the aforementioned step (a), for selecting surviving cells in a selection medium as ES-like cells.

40. The selection method of claim 39, wherein mutually

different drug resistance genes are present at the positions permitting expression control by the expression control regions of the ECAT2 gene and the ECAT3 gene.

41. The selection method of claim 33, which comprises the following steps (a) and (b):
(a) a step for bringing into contact with each other a somatic cell comprising a gene wherein a drug resistance gene is present at a position permitting expression control by the expression control region of the ECAT4 gene, and a somatic cell nuclear reprogramming substance,
(b) a step following the aforementioned step (a), for selecting surviving cells in a selection medium as ES-like cells.

42. A use of the somatic cell of any of claims 26 to 32 in the screening method of any of claims 1 to 16 or the selection method of any of claims 33 to 41.

43. A cell expressing the marker gene or surviving cell that has emerged in the screening method of any of claims 1 to 16, or an ES-like cells selected in the selection method of any of claims 33 to 41.

44. A screening method for a substance for the maintenance of undifferentiated state and pluripotency of ES cells, which comprises the following steps (a) and (b):
(a) a step for bringing an ES cell comprising a gene wherein a marker gene is present at a position permitting expression control by the expression control region of an ECAT gene into contact with a test substance in a medium not allowing the maintenance of undifferentiated state and pluripotency of ES cells,
(b) a step following the aforementioned step (a), for determining the presence or absence of cells expressing the marker gene, and selecting a test substance allowing the occurrence of the cells as a candidate substance for the

maintenance of undifferentiated state and pluripotency of ES cells.

45. The screening method of claim 44, wherein the ECAT gene is one or more genes selected from among the ECAT1 gene, ECAT2 gene, ECAT3 gene, ECAT4 gene, ECAT5 gene, ECAT6 gene, ECAT7 gene, ECAT8 gene, ECAT9 gene and Oct3/4 gene.

46. The screening method of claim 44 or 45, wherein the marker gene is a drug resistance gene, a fluorescent protein gene, a luminescent enzyme gene, a chromogenic enzyme gene or a gene comprising a combination thereof.

47. The screening method of any of claims 44 to 46, wherein the ES cell is an ES cell comprising a gene resulting from knocking in a marker gene to an ECAT gene.

48. The screening method of claim 47, wherein the ES cell is an ES cell homozygously comprising a gene resulting from knocking in a marker gene to an ECAT gene.

49. The screening method of claim 47 or 48, wherein the ECAT gene is one or more genes selected from among the ECAT1 gene, ECAT2 gene, ECAT3 gene, ECAT4 gene, ECAT5 gene, ECAT6 gene, ECAT7 gene, ECAT8 gene, ECAT9 gene and Oct3/4 gene.

50. The screening method of claim 44, which comprises the following steps (a) and (b):

(a) a step for bringing an ES cell comprising a gene resulting from knocking in a gene comprising a drug resistance gene to the ECAT2 gene into contact with a test substance in a medium not allowing the maintenance of undifferentiated state and pluripotency of ES cells,

(b) a step following the aforementioned step (a), for determining the presence or absence of surviving cells in a selection medium, and selecting a test substance allowing the

occurrence of the surviving cells as a candidate substance for the maintenance of undifferentiated state and pluripotency of ES cells.

51. The screening method of claim 44, which comprises the following steps (a) and (b):
(a) a step for bringing an ES cell comprising a gene resulting from knocking in a gene comprising a drug resistance gene to the ECAT3 gene into contact with a test substance in a medium not allowing the maintenance of undifferentiated state and pluripotency of ES cells,
(b) a step following the aforementioned step (a), for determining the presence or absence of surviving cells in a selection medium, and selecting a test substance allowing the occurrence of the surviving cells as a candidate substance for the maintenance of undifferentiated state and pluripotency of ES cells.

52. The screening method of claim 44, which comprises the following steps (a) and (b):
(a) a step for bringing an ES cell comprising a gene resulting from knocking in a gene comprising a drug resistance gene to the ECAT5 gene into contact with a test substance in a medium not allowing the maintenance of undifferentiated state and pluripotency of ES cells,
(b) a step following the aforementioned step (a), for determining the presence or absence of surviving cells in a selection medium, and selecting a test substance allowing the occurrence of the surviving cells as a candidate substance for the maintenance of undifferentiated state and pluripotency of ES cells.

53. The screening method of claim 44, which comprises the following steps (a) and (b):
(a) a step for bringing an ES cell comprising genes resulting from knocking in a gene comprising a drug resistance gene to

each of the ECAT2 gene and the ECAT3 gene into contact with a test substance in a medium not allowing the maintenance of undifferentiated state and pluripotency of ES cells,
(b) a step following the aforementioned step (a), for determining the presence or absence of surviving cells in a selection medium, and selecting a test substance allowing the occurrence of the surviving cells as a candidate substance for the maintenance of undifferentiated state and pluripotency of ES cells.

54. The screening method of claim 53, wherein the different drug resistance genes have been knocked in to ECAT2 gene and the ECAT3 gene.

55. The screening method of any of claims 50 to 54, wherein the ES cell is an ES cell homozygously comprising a gene resulting from knocking in a gene comprising a drug resistance gene to an ECAT gene.

56. The screening method of claim 44, which comprises the following steps (a) and (b):
(a) a step for bringing an ES cell comprising a gene resulting from knocking in a gene comprising a drug resistance gene to the ECAT4 gene into contact with a test substance in a medium not allowing the maintenance of undifferentiated state and pluripotency of ES cells,
(b) a step following the aforementioned step (a), for determining the presence or absence of surviving cells in a selection medium, and selecting a test substance allowing the occurrence of the surviving cells as a candidate substance for the maintenance of undifferentiated state and pluripotency of ES cells.

57. The screening method of claim 56, wherein the ES cell is an ES cell heterozygously comprising a gene resulting from knocking in a gene comprising a drug resistance gene to the

ECAT4 gene.

58. A substance for the maintenance of undifferentiated state and pluripotency of ES cells selected using the screening method of any of claims 44 to 57.

59. The substance for the maintenance of undifferentiated state and pluripotency of ES cells of claim 58, which is a secretion product of feeder cells.

60. The substance for the maintenance of undifferentiated state and pluripotency of ES cells of claim 58, which is a serum-derived component.

61. A use of a knock-in mouse comprising a gene resulting from knocking in a marker gene to an ECAT gene as a source of the ES cell used in the screening method of any of claims 44 to 57.

62. The use of claim 61, wherein the knock-in mouse is a knock-in mouse homozygously comprising a gene resulting from knocking in a marker gene to an ECAT gene.

63. The use of claim 61 or 62, wherein the ECAT gene is one or more genes selected from among the ECAT1 gene, ECAT2 gene, ECAT3 gene, ECAT4 gene, ECAT5 gene, ECAT6 gene, ECAT7 gene, ECAT8 gene, ECAT9 gene and Oct3/4 gene.

64. The use of any of claims 61 to 63, wherein the marker gene is a drug resistance gene, a fluorescent protein gene, a luminescent enzyme gene, a chromogenic enzyme gene or a gene comprising a combination thereof.

65. An ES cell comprising a gene wherein a marker gene is present at a position permitting expression control by the expression control region of an ECAT gene.

66. The ES cell of claim 65, wherein the ECAT gene is one or more genes selected from among the ECAT1 gene, ECAT2 gene, ECAT3 gene, ECAT4 gene, ECAT5 gene, ECAT6 gene, ECAT7 gene, ECAT8 gene, ECAT9 gene and Oct3/4 gene.

67. The ES cell of claim 65 or 66, wherein the marker gene is a drug resistance gene, a fluorescent protein gene, a luminescent enzyme gene, a chromogenic enzyme gene or a gene comprising a combination thereof.

68. The ES cell of any of claims 65 to 67, which comprises a gene resulting from knocking in a marker gene to an ECAT gene.

69. The ES cell of claim 68, which homozygously comprises a gene resulting from knocking in a marker gene to an ECAT gene.

70. A use of the ES cell of any of claims 65 to 69 in the screening method of any of claims 44 to 57.